

Award Number:

W81XWH-10-2-0129

TITLE: Homeostatic and Circadian Abnormalities in Sleep and Arousal in
Gulf War Syndrome

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REPORT DATE: October 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) October 2016		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 20 Sept2015 to 19 Sept2016	
4. TITLE AND SUBTITLE Homeostatic and Circadian Abnormalities in Sleep and Arousal in Gulf War Syndrome				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-10-2-0129	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Timothy M Juergens MD Giulio Tononi MD Ruth Benca MD PhD Email: timothy.juergens@va.gov				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Wisconsin System Board of Regents 21 N Park ST STE 6401 Madison, WI 53715-1218				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The purpose of this study is to assess sleep and wake parameters in veterans of the first Gulf War who have fatigue and other symptoms compared with veterans who do not have fatigue utilizing novel assessment techniques including high density EEG and temperature. This research study is in the data collection and data processing phase. The most significant findings to date in this study during the research period include continued evidence of high density EEG marked broad band reduction in neural activity circumscribed in the frontal cortex in NREM sleep. Slow wave sleep is considered to play a role in the recovery and restorative aspects of sleep, and is one bandwidth affected. In addition, melatonin curves, particularly dim light melatonin onset, which is well-tied with circadian sleep/wake and subsequent feelings of fatigue/alertness show different projections in veterans endorsing fatigue than those who do not.					
15. SUBJECT TERMS Dense array EEG, temperature, melatonin, vigilance					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	9	19b. TELEPHONE NUMBER (include area code)

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Introduction

This research project assesses sleep and wake parameters in veterans of the first Gulf War who have fatigue and other symptoms compared to veterans who do not have fatigue. It utilizes novel assessment of brain waves with high density EEG. This tool allows for high spatial as well as temporal resolution to provide a window into how sleep is regulated at the global and local level. This will allow us to determine how specific sleep pattern activity is altered in veterans with fatigue. Beyond the typical overnight polysomnography, this assessment includes objective wave analysis of slow wave characteristics, origin and propagation. Circadian rhythm is also assessed, including temperature and salivary melatonin measures, as well as salivary cortisol levels. Vigilance at various points is tested with a psychomotor vigilance test, and there is an optional genetic testing part of the study to assess many polymorphisms that have been associated with other fatiguing conditions and symptoms.

Body

In the Statement of Work, we anticipated being in the recruitment and running subjects in the protocol phase, and we have successfully completed all recruitment and subject appointments. We particularly focused on our control group and completed 6 additional subjects for a total of 22 as planned. This recruitment target was met by numerous increased recruitment efforts.

Data collected includes core, peripheral and distal body temperature, two nights of dense array EEG, multiple symptom scales involving fatigue, pain, and other symptoms, cortisol samples to be able to note diurnal changes, as well as morning cortisol rise from natural wake. We also have collected melatonin samples in a low light environment to be able to assess dim light melatonin onset. Psychomotor vigilance task (PVT) data has been collected at various points in the day in concert with subjective fatigue and sleepiness data.

OVERNIGHT PSG REPORT

Comparisons of our fatigued veteran age-matched healthy control subjects on standard polysomnographic parameters is noted. These include variables such as respiratory events (apneas and hypopneas), time spent in each sleep stage (N1, N2, N3), sleep efficiency, total sleep time, REM duration and latency, wake after sleep onset, and arousals. Despite differences in sleep topography, there were not differences in any polysomnographic parameter between groups, although there was a trend toward a *higher* arousal index in the control subjects. Final analysis of such will come on future reports. These data, along with those presented below, suggest that standard PSG metrics are not sufficient to capture the subtle, but physiologically important changes in sleep in subjects with fatigue.

EEG ANALYSIS

As previously reported, we have conducted comparisons of all night spectral power as well as sleep topography in our fatigued veterans versus healthy control subjects.

Comparisons of the all-night power spectra demonstrate a slight increase in high-frequency activity (28-32 Hz) in the GWI group relative to the healthy control subjects, although no differences were observed in low frequency activity.

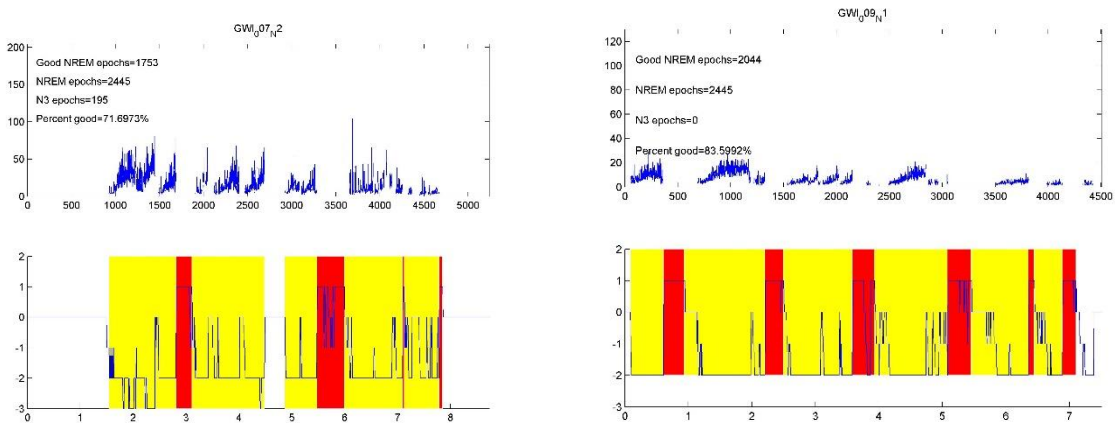


Figure 1: Comparison of all night data between symptomatic subject (left) and control (right).

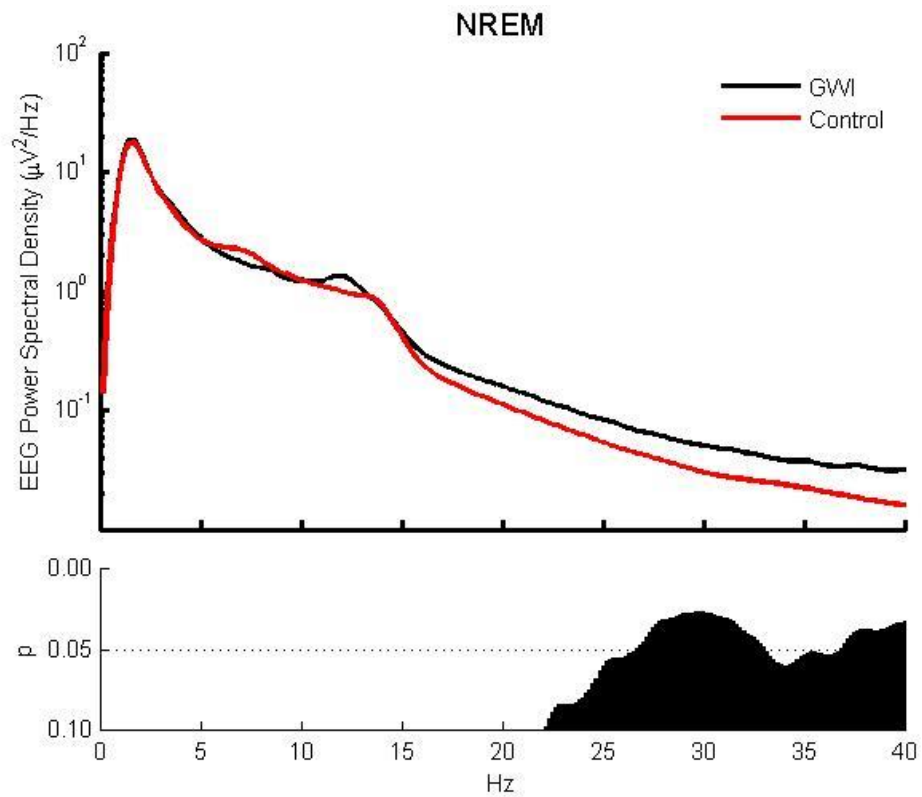


Figure 2: All night power spectral analysis (shown prior)

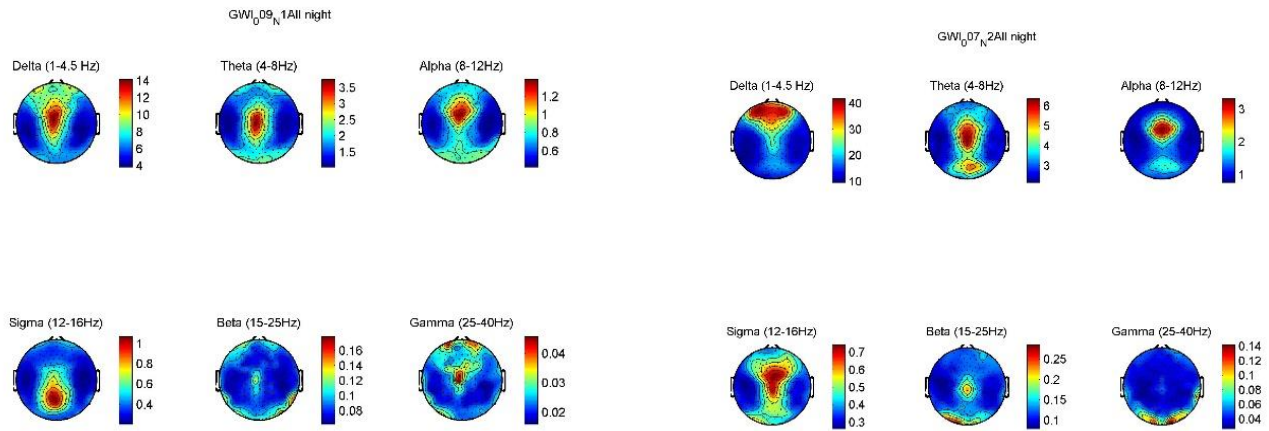


Figure 3: Absolute Non REM topical distribution of spectral frequency analysis comparing active subject (left) with control subject (right). Delta slow wave frontal activity in this example shows more prominent in the control subject, versus active group though overall these differences were minimal.

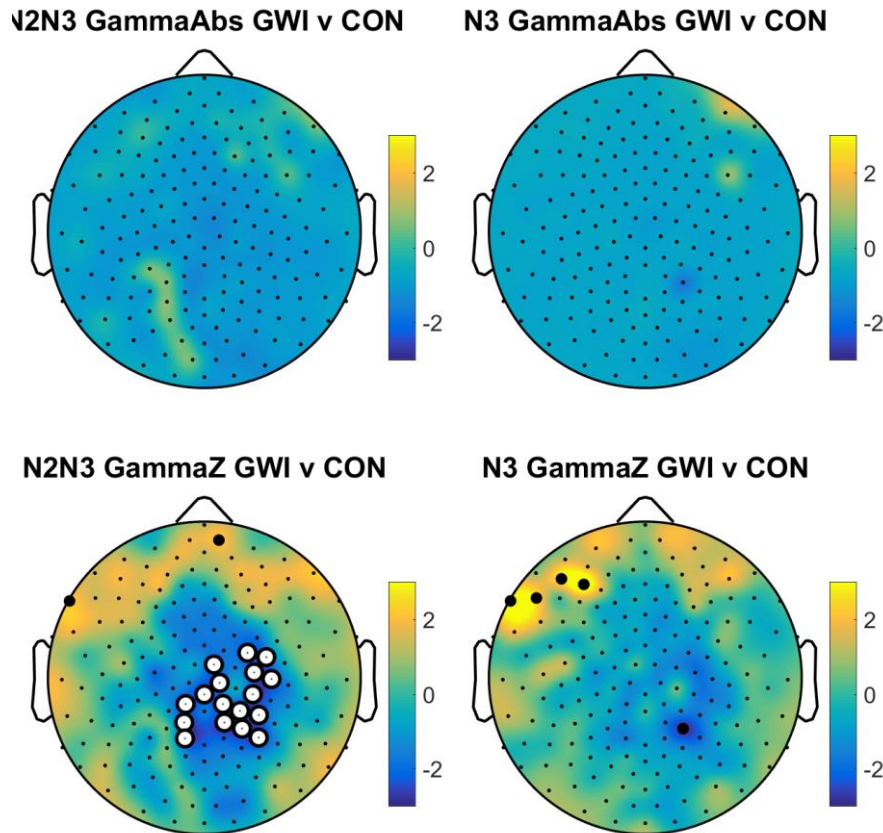


Figure 4: Higher frequency (Gamma 28-40hz) differences with some increased high frequency activity in active versus control veterans.

The difference in low frequency (delta) frontal activity was noted to be quite prominent when comparing active subjects to a 2nd EEG healthy control group who had not (below).

NREM SWAZ GWI v CON

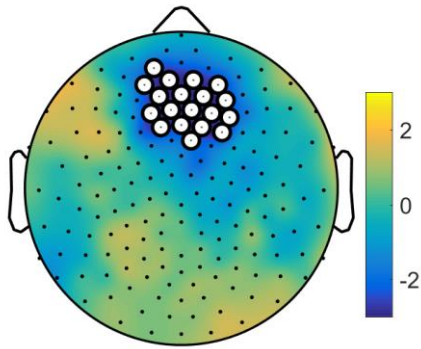


Figure 5: Slow wave frontal activity is significantly decreased in active subjects compared to an EEG control group of healthy subjects.

REM SWAZ GWI v HControls2

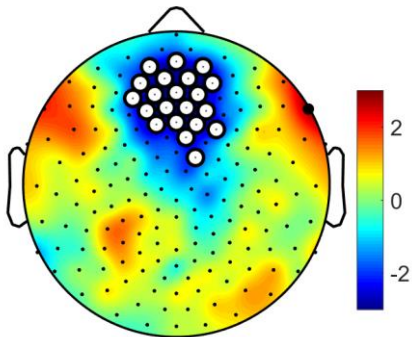


Figure 6: This frontal hypoactivity is also noted during REM sleep in comparing the same two groups.

It is established that sleep itself is not a global phenomenon, but occurs in a regional/localized, often use-dependent manner.¹⁻⁴ One explanation for the daytime fatigue and cognitive impairments commonly reported in GWI may be that these veterans undergo frontally specific sleep deprivation. This frontal area is well-known to impact cognitive function. Since sleep plays a central role in learning and performance, a failure of sleep-related oscillations, particularly SWA, to encompass frontal areas would have deleterious impacts on short-term daytime function. Moreover, optimal sleep is not only critical for daytime learning and performance, but emerging evidence indicates that low frequency sleep slow-waves play a critical role in regulating cortical plasticity.⁵

An alternative but related interpretation of these data is that it is a reflection of neural injury in this cortical region, arising either as a consequence of long-term sleep loss or as a result of an unknown process related to Gulf-War participation. The notion that sleep pathology results in acute impairments of frontal lobe function has long-standing support, but more recent data in the literature suggests that detriments in sleep may ultimately impact the structural integrity of the frontal lobe. A recent volumetric analysis of Gulf-War veterans, adults with major depression and those with PTSD demonstrated reductions in frontal lobe volume associated specifically with poor sleep quality.⁶ Grey matter reductions in prefrontal cortex have been reported in patients with clinical sleep disorders, including insomnia and cataplexy.⁷⁻⁹ A recent hdEEG analysis of obstructive sleep apnea in men of a similar age to our subjects in this study, identified a circumscribed reduction in neural activity over the posterior parietal cortex across all frequencies. Given that many neuroimaging modalities indicate the presence of neural injury the posterior cortex in patients with this disorder, the authors interpreted

these data as offering further support of neural injury.¹⁰ One question we are still looking at in our data is that in comparing even our deployed control group to a group of healthy controls, we are finding some similar differences, raising concern that this deployed group overall may be exhibiting some of these EEG phenomenon.

Melatonin data, while still being processed appears to be showing similar overall levels between groups, but a more marked slope of increased melatonin levels over the hours immediately prior to sleep in the control group compared to the active group. This would be consistent with some impact from a more robust circadian rhythm in the control group.

Now that we have completed subject recruitment, we are in the process of correlating this functional deficit on EEG with other parameters, including temperature anomalies in this group of subjects to determine if the effect may be related to alterations in the phase relationships between sleep and temperature rhythms. If so, this would support the notion that subjects with GWI are in a state of circadian misalignment, which could also account for many of the symptoms to the syndrome, including cognitive impairment, gastrointestinal distress and fatigue. The addition of our control subjects we completed and are adding to the data analysis will give the most robust opportunity for comparison, and help direct whether the noted EEG changes are related to circadian rhythm abnormalities or more to homeostatic sleep process issues.

Key Research Accomplishments

- Completed recruiting
- Data collection
- Data processing
- Some data analysis

Reportable Outcomes

We submitted and were accepted to present the EEG data differences at the American Academy of Sleep Medicine Conference in June 2016 through a poster presentation. Additional reportable outcomes have not yet occurred. We are currently in the data processing and assessment, as well as initial write up of EEG findings for publication.

Conclusion

Although firm conclusions are premature, we have demonstrated that, despite the appearance of adequate nocturnal sleep, there are marked differences in the frontal profile of sleep in veterans of the Gulf-War relative to healthy control subjects. Whether this nighttime pattern of frontal disruption is a consequence of neural injury or if it is a reflection of poor quality sleep is unclear. Long-term sleep disruption is associated with alterations in the structural integrity of the frontal cortex, alterations that may arise secondary to impairments in the neural plasticity that are known to occur during sleep. Examinations of functional EEG activity during the daytime may help to clarify whether this is a 24-hour phenomenon or something more impacting sleep. Regardless, this sleep-related deficit could surely explain some of the cognitive symptoms associate with GWI as well as related fatigue.

These finding offers some potential areas of future targeted treatments. Other potential contributors will continue to be assessed when they are analyzed (batched), including cortisol and subject samples of melatonin.

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